A fourth locus for Autosomal Dominant Hypercholesterolemia maps at 16q22.1

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Abstract

Autosomal Dominant Hypercholesterolemia (ADH) is characterized by isolated elevation of plasmatic LDL cholesterol associated with high risk of premature cardiovascular disease. Mutations in *LDLR*, *APOB* and *PCSK9* genes have been shown to cause ADH. We now report further genetic heterogeneity of ADH through the study of a large French family in which the involvement of theses three genes was excluded. A genomewide scan mapped the disease-causing gene, named *HCHOLA4*, at 16q22.1 in a 7.89 Mb interval containning 154 genes with a maximum lod-score of 3.9. To reduce the linked region, we genotyped 18 smaller non*LDLR*/non*APOB*/non*PCSK9*-ADH families at the *HCHOLA4* locus. Six families did not exclude linkage to the locus, but none allowed reduction of the disease interval. The 154 regional genes were sorted according to the function of the encoded protein, and tissue expression profiles, and 57 genes were analysed through sequencing of their coding region and close flanking intronic parts. No disease-causing mutation was identified in these families, particularly in the *LCAT* gene. Finally, our results also show the existence of other ADH genes since 9 families were neither linked to the *LDLR*, *APOB*, *PCSK9* genes nor the new *HCHOLA4* locus.

Key Words: hypercholesterolemia, genetics, mapping, lipoprotein metabolism

Autosomal co-Dominant Hypercholesterolemia (ADH, OMIM #143890) is one of the most frequent inherited disorders in man with a frequency of 1/500 for heterozygotes in Western populations. It is characterized by a selective increase of Low Density Lipoprotein (LDL) particles in plasma giving rise to tendon and skin xanthomas, arcus cornea, and premature mobidity and mortality from cardiovascular complications. This disease has proven to be genetically heterogeneous and associated with defects in 2 different genes: *LDLR* (low density lipoprotein receptor) (1,2), and *APOB* (apolipoprotein B-100) (3). Our team pioneered the hypothesis that the disease was genetically more heterogeneous. We mapped a third major locus at 1p34.1-p32(4) and went on to show that it encodes PCSK9 (Proprotein Convertase Subtilisin/Kexin type 9) (5). Altogether, we identified 7 mutations in 8 families (5,6, JPR personal data) and showed that mutations in the *PCSK9* gene account for only 1.8 % of gene defects in a sample of 392 ADH families studied in our laboratory. Subsequently, Cohen et al. showed that mutations within the gene are also involved with a dominant form of hypocholesterolemia (7). Targets of PCSK9 convertase are still unknown and its biological function is still not clear, although it is now known to enhance the degradation of the low density lipoprotein receptor (8).

While investigating ADH families, we identified a group of families neither harboring mutations within the three major genes nor linked to any of these genes.We now report one of these families, a large French pedigree, in which we postulated the involvement of a fourth gene we named *HCHOLA4*. The objective of this study was to localize and possibly identify ADH disease loci additional to the three major loci known.

Materials and Methods

Familly recruitment and disease ascertainment

Hypercholesterolemic families were recruited by the French National Research Network on Hypercholesterolemia. For probands, the following selection criteria were used: total cholesterol and apoB values above the 95 th percentile when compared to a sex- and age-matched French population (personal data from Institut Régional pour la Santé, Tours-La Riche, France), triglycerides below 1.5 mmol/L, personal and/or documented familial xanthomas, xanthelasmas, and/or arcus corneae, and early coronary artery disease. Lipid measurements were repeated to ascertain the existence of primary type IIa hypercholesterolemia. Families were investigated at large to confirm the presence of ADH. Blood samples were obtained from the index patient and his, her relatives for isolation of DNA. The study was performed in accordance with French bioethic regulations and written informed consent was given by all subjects, and when appropriate, by parents or guardians.

Genomewide scan and linkage analysis

A genomewide scan was performed using 232 polymorphic microsatellite markers spaced at 20 cM intervals on autosomes (9). All marker-typing data were collected blindly and independently by four of us. A second genomewide scan was performed using a GeneChip® 10K Array (Affymetrix chips, GeneChip® Human Mapping 10K Array Xba 142) in collaboration with "Centre National de Génotypage" (Evry, France). We used parametric linkage in two situations: the first, an "affected-only" analysis where all related family members were given either an affected or an unknown disease status; the second, a "whole family" analysis where all related familly members were given an affected, unaffected or unknown status as provided by referring clinicians and in agreement with clinical and biological data. Parametric linkage analyses were performed with the following parameters:

(1) autosomal dominant transmission of the disease trait, (2) reduced penetrance of 0.9 for heterozygotes (or complete penetrance for the affected-only analyses) and (3) frequency of the disease allele of 0.004% (close to the frequency of *PCSK9* mutants). The power of the families for linkage was evaluated using the FastSlink v2.51 program (10). We used Pedcheck (11) to detect Mendelian inheritance errors. SuperLink v1.5 and SimWalk v2.91 programs were used to compute two-point and multipoint LOD scores (12,13). All these programs were run with the easyLinkage Plus v5.00 package (14). Additional model-free analyses were realized with the GENEHUNTER program that performs a complete multipoint to infer the degree of identity-by-descent sharing among all affected family members at each map point (15). We used GENEHUNTER-PLUS, a modified version of this program , that has been shown to be less conservative, particularly when data are less than perfectly informative (16). This program calculates a semi-parametric LOD score (ghp-LS) by using a single parameter that is the measure of the inheritance vector in the pedigree and allele sharing.

Candidate genes investigation

DNA was extracted from venous blood using a technique described by Collod et al.(17). A selection of 57 regional candidate genes was sequenced (coding regions and close flanking intronic parts) as previously reported (5). Primer sequences are available on request.

LCAT activity measurement

Lecithin Cholesterol Acyltransferase (LCAT) activity in the plasma was measured by a non-radioactive endogenous cholesterol esterification method (18). Briefly, it consists in measuring the decrease in plasma free cholesterol content following incubation at 37°C.

Results

Identification of a nonLDLR/nonAPOB/nonPCSK9 ADH Family

Among 69 consecutive nonLDLR/nonAPOB/nonPCSK9 families identified in our laboratory, one family was large enough to enable genomewide mapping.The recruitment of the HC6 family was initiated through the female proband II-4 who presented a total cholesterol value of 3.64 g/L (9.39 mmol/L) at 54 years of age without medication. We enlarged the family to 30 individuals over 4 generations, thus exploring 23 meioses (Table 1 and, Figure 1). Several cardiovascular accidents were reported in the previous generations. Clinical examination of affected members revealed no xanthoma, xanthelasma or corneal arcus. Carotid intimal-medial thickness was elevated for patient II-1 whereas it was normal for his sister II-4 (data not shown). Ten family members presented elevated blood cholesterol with isolated hyperLDLemia; 13 family members and 5 spouses showed no clinical or biological anomaly and were considered non affected. Finally, a father II-9 and son (III-14) were scored as "unknown" for linkage analysis because of high triglycerides (2.53 mmol/L) in the father. Distributions of total and LDL cholesterol values in all tested family members were bimodal, thus compatible with an autosomal dominant transmission of the disease (data not shown). The candidate gene approach excluded genetic linkage between the ADH phenotype and any of the three ADH-causing genes identified so far. Indeed, regional haplotypes constructed in the HC6 family for polymorphic markers covering *LDLR* (19p13.2), *APOB* (2p24.1), and *PCSK9* (1p32.3) genes showed no cosegregation of a particular allelic association with the ADH phenotype. Furthermore, with a "whole family" linkage analysis, exclusion lod score values of - 4.95, -3.47 and - 2.49 were obtained for the *LDLR*, *APOB* and *PCSK9* genes, respectively. Finally, no deleterious mutation was observed after sequencing the *LDLR*, *APOB*, and *PCSK9* genes, and no major rearrangement was found in the *LDLR* gene (data not shown).

Linkage analysis

One hundred thousand simulations were performed to evaluate the power and the relevance of a genomewide linkage scan in the HC6 family. In the "affected-only" analysis, the average expected LOD score was 1.60 and the maximum expected LOD score was 2.41. In the "whole familly" analysis, the average expected LOD score was 3.18 and the maximum expected LOD score was 4.81, indicating that the statistically significant threshold of 3 could be reached with this single family with 51.3% of maximum lod scores greater than 3.3 (empirical p=0.010), and for thresholds of 3.7 or 3.9, empirical p= 0.001. Among the 232 microsatellite markers initially tested in the "whole familly" analysis, seven had LOD scores above 1.2: markers D8S263 (LOD=1.41, θ=0), D10S186 (LOD=1.29, θ=0), D12S310 (LOD=1.43, θ=0.15), D12S87 (LOD=1.32, θ=0.2), D16S3107 (LOD=3.77, θ=0), D16S515 (LOD=1.36, θ=0) and D20S200 (LOD=1.38, θ=0.15).

To confirm and further investigate possible linkage in these regions, 500 SNPs as well as 12 additional microsatellite markers were used. Convincing exclusion data were obtained for chromosomes 8, 10, 12 and 20. At 16q22, seven additional microsatellite markers and 100 SNPs of the 16q22 region were tested to saturate and to delimit a disease interval. Results of this analysis showed high LOD scores for nine of the new markers (Table 2 and Supplemental Table 1). In the "affected-only" analysis, five markers, D16S3031, rs725131, D16S3107, D16S3067 and D16S3018, had $LOD_{\theta=0}$ scores of 2.11, 1.85, 2.41, 2.41 and 1.98, respectively (Supplemental Table 1). In the "whole family" analysis, $LOD_{\theta=0}$ scores for rs725131, D16S3107, D16S3067 and D16S3083 raised to 3.36, 3.77, 3.91 and 2.41, respectively, whereas the maximum LOD score for D16S3031 was reduced to 1.46 at θ =0.10 (Table 2).

To investigate whether 16q22.1 linkage could result from the use of an inaccurate model, we performed model-free analyses with GENHUNTER-PLUS (from marker D16S3043 to

D16S518) in affected individuals of the HC6 family. A semi-parametric LOD score (ghp-LS) was calculated. The ghp-LS was null at markers D16S3043 and D16S518 thus excluding linkage to these two markers. The ghp-LS was positive and maximal (1.63) from markers D16S3031 to D16S515 (data not shown).

Regional haplotype construction allowed the identification of a common region for all affected members from D16S3043 to D16S518 (Figure 1). Indeed, recombinational events were oberved in patient II-5 for the proximal border (between D16S3043 and D16S3031) and in patient II-1 for the distal border (between rs254770 and D16S518). Non-affected recombinants allowed reduction to a minimum interval of 8.39 Mb between markers D16S3031 and D16S3018 (Figure 1). Indeed, recombinational events were found in members II-11 and III-4 for the proximal border (between D16S3031 and rs725131) and in member III-10 for the distal border (between D16S3067 and D16S3018). Subject III-11 (non-affected) also inherited the disease-associated haplotype and thus highly probably displays incomplete penetrance of the HCHOLA4 disease. Phenotypically unclassified patients (II-9 and III-14) did not carry the disease haplotype. The regional multipoint LOD score analysis gave a maximum value of 3.81 and allowed the identification of a minimum interval of 7.89 Mb between 64 687 100 and 72 127 100 bp at 16q22 (Figure 2).

To reduce the linked region, we genotyped polymorphic markers of the *HCHOLA4* locus in 18 ADH families that showed exlusion of the *LDLR*, *APOB* and *PCSK9* genes as well as the absence of mutation within these genes (Table 3). These 18 families represented a total of 75 meioses with 74 affected and 49 non-affected subjects. Three families (HC32, HC122 and HC218) were non informative. Nine families (HC14, HC35, HC42, HC73, HC126, HC138, HC257, HC374 and HC438) showed exclusion of the locus. The six remaining families (HC38, HC49, HC120, HC136, HC205 and HC266) did not exclude linkage to the *HCHOLA4* locus but were not large enough to provide significant linkage. Furthermore, none

of them showed recombinational events in the disease interval. In effect, the recombinational event found in family HC136 occured between markers D16S3031 and D16S3019 (position 64 686 681 bp) (data not shown), and thus was distal to the regional boundary obtained with subject III-4 from the HC6 family, that occured between markers D16S3031 and rs725131 (position 65 143 211 bp) (Table 2, Figure 1).

Analysis of regional candidate genes

An inventory of all the genes and ORFs present between these two positions was drawn up using data from the UCSC Genome Browser (http://www.genome.ucsc.edu/cgi-bin/hgGateway), the Genatlas database (http://www.dsi.univ-paris5.fr/genatlas/) and the Ensembl Database (http://www.ebi.ac.uk/ensembl/). This study showed that the disease interval contains 154 genes (Supplemental Table 2). The first gene investigated was the *LCAT* gene since it encodes a major actor of cholesterol metabolism. No mutation was identified in affected members of the HC6 family after sequencing of the 6 exons and intronic flanking regions of the gene. Furthermore, LCAT enzymatic activity was tested in two affected members of the HC6 family (subjects II-4 and III-5) and was not significatively different from that of controls (82.30 ±5.59 versus 70.00 ±3.54 nmol CE/h/mL of plasma for controls, $p = 0.07$).

Several genes within the locus were good candidates and we defined a priority for analysis according to the function of the encoded proteins and their tissue expression. We tested the coding sequence of 57 candidate genes by direct sequencing (Supplemental Table 2). No deleterious variation was identified. However, this systematic sequencing allowed the identification of new polymorphisms that were used to comfort regional haplotypes, and to reduce the common interval. We identified one polymorphism in *C16orf48* (c.780A>G). Its frequency was estimated at 0.49 by direct sequencing of 60 healthy individuals. All carriers of

the affected haplotype in the HC6 family shared this polymorphism. A two-point linkage analysis gave a LOD score of 3.52 (θ=0). Interestingly, this gene is located close to marker D16S3067 for which we obtained the highest regional LOD score.

Discussion

In the present study, a genomewide scan performed in a single large French family mapped a fourth ADH gene (different from *LDLR*, *APOB* and *PCSK9*) at 16q22.1. The highest LOD scores of 3.77 (θ=0) and 3.36 (θ=0) were obtained for markers D16S3107 and rs725131, respectively, with the "whole familly" analysis to maximize the number of investigated meioses. Mapping was confirmed by a multipoint analysis that gave significative LOD scores as well. Furthermore, no other region of the genome showed LOD score values as important as these. Haplotype construction in the HC6 family allowed delineation of a critical region between markers D16S3043 and D16S518 considering only affected members, and between markers D16S3031 and D16S30106 when unaffected individuals were also taken into account. Subject III-11, who carries the disease-associated haplotype but presents a normal phenotype, may illustrate the well documented incomplete penetrance of ADH. Indeed, there is an extreme variability of the hypercholesterolemic phenotype with well documented cases of incomplete penetrance probably due to the effect of modifier factors. For example, Sass et al. reported a French Canadian family with a 5 kb deletion in the *LDLR* gene with several carriers of the mutation presenting normal cholesterol values (19). In the same way Hobbs et al. described a large Puerto Rican family carrying the p.Ser156Leu (now p.Ser177Leu according to the international nomenclature) mutation of the *LDLR* gene (20). In this family, penetrance of the disease could be estimated at 0.68 (13/19), since six subjects were non-manifesting carriers of the mutation. As well, in our first report of the p.Ser127Arg mutation

in the *PCSK9* gene, one carrier of the mutation displayed normal cholesterol levels contrary to the other family members carriers of the disease-causing mutation who were hypercholesterolemic (5).

To reduce the linked region, 18 smaller ADH families that showed exlusion of the involvement of the *LDLR*, *APOB* and *PCSK9* genes were studied. Only 6 did not exclude linkage to the *HCHOLA4* locus but were not large enough to independantly provide significant linkage. Furthermore, no recombinational event was detected that would have allowed the reduction of the disease interval. Recruitment of new family members and new families is now necessary to replicate significant linkage and to define a smaller inteval.

None of the recent genomewide association or linkage studies (21-25) identified any loci fulfilling our two search criteria: 1) affecting LDL (only lipoprotein elevated in the HCHOLA4 families), and 2) localized within positions 64 687 100 – 72 127 000 bp at 16q22. Three genomewide association studies established an association between loci localized within the HCHOLA4 locus at 16q22.1 reported here, and variation in HDL concentrations (21-23). These loci are generally considered to reflect association of HDL levels with the *LCAT* gene. Mutations in the *LCAT* gene are associated with Fish Eye disease (OMIM #136120). Fish Eye disease is characterized by normal serum cholesterol and raised serum triglycerides, VLDL, and LDL triglycerides whereas HDL cholesterol is reduced. Even though Fish Eye disease and ADH present different phenotypes, we explored the *LCAT* gene as a HCHOLA4 candidate because of its involvement in cholesterol metabolism. Unsurprisingly, no mutation was found in the affected subjects of the HC6 family. Furthermore, LCAT enzymatic activity of two affected family members was not significantly different from that of controls. Thus, involvement of the *LCAT* gene was clearly excluded.

Our results reveal the existence of a new partner of either LDL receptor-mediated endocytosis or intracellular trafficking, or even a new and different endocytic pathway. .Better knowledge

has been gained in recent years on the molecular basis of transmembrane traffic of cargo proteins. The clathrin-dependent pathway, involved in the endocytosis of receptor-bound LDL particles, is now known to involve adaptors (heterotetrameric protein complexes or AP2 complexes) that are responsible for cargo sorting (27). However other traffic pathways have been defined either associated with coated vesicles (COPI and COPII) or caveolae (28). Also noteworthy is the protein encoded by the recently cloned *ARH* gene (MIM #605747). Its protein product called LDLR adaptor protein 1contains a phosphotyrosine binding domain that is also found in adaptator proteins and that could bind the cytoplasmic NPXY motif of the LDL receptor. Mutations of the *ARH* gene are found in patients presenting with the autosomal recessive form of familial hypercholesterolemia (29). In this context, several genes at 16q22.1 were also excellent functional candidates. Among these, 5 genes were studied first:

- The *AP1G1* gene encodes the adaptor-related protein complex 1, gamma-1 that belongs to the adaptor complexes large subunit family, involved in vesicles transport from the trans-Golgi network to lysosomes (30).
- The *VPS4A* gene encodes a AAAtype ATPase belonging to the family of vacuolar sorting proteins originally identified in yeast. Genetic studies led to the identification of a subset of yeast “vps” mutants that accumulate an exagerated late endosome known as the “class E” compartment. These mutants were found to be defective in multivesicular body formation (31).
- The *RRAD* gene encodes a ras-related GTPase already involved in diabetes and that might be involved in LDL receptor endocytosis since GTPase Rho plays a role in cellular uptake of LDL by human skin fibroblasts (32).
- The *ATP6V0D1* gene (vesicular ATPase), encodes a vacuolar proton-ATPase and is possibly involved in the transport from late endosome to lysosomes of membrane proteins (33).

- The *FLJ12076* gene encodes a homolog of LIN-10 involved in EGF receptor (LET-23) membrane localization in *C. elegans* (34). LIN-10 protein contains a PDZ domain known to interact with cytoplasmic tails of membrane proteins (35).

No mutation was found in any of these genes nor among the 52 other regional genes that we studied. However, this does not totally exclude the possibility that one may very well be the causative gene since only exons and their flanking intronic regions were sequenced.

The present study gives evidence of the existence of a new gene involved in the pathogenesis of ADH. The existence of a greater level of genetic heterogeneity is in agreement with recent reports since the proportion of ADH subjects without an identified mutation ranges from 12% to 72% depending on the study (36). Such a large difference in mutation identification is probably due to different sample sizes, to heterogeneous clinical definitions and screening protocols. Overall, the best estimated proportion of individuals without a mutation in any of the three identified ADH genes is 15.25% (36). This group of new forms of ADH is very probably itself heterogeneous and the proportion of *HCHOLA4*-affected individuals may not be more important than that of *PCSK9* carriers that we estimated at 1.5 % (36). *HCHOLA4*-linked ADH may thus be considered as a very rare form of ADH. Whatever its function, the HCHOLA4 protein may be a player in the pathway that involves the convertase PCSK9 and thus be also involved in the intracellular traffiking of the LDL receptor. If so, its identification would help to elucidate the pathophysiology of both *PCSK9*-linked and *HCHOLA4*-linked ADH. Furthermore, as a novel protein implicated in the regulation of cholesterol metabolism, HCHOLA4 might constitute a new target for hypocholesterolemic treatment.

In conclusion, we report the detection and chromosomal localization of the fourth gene involved in ADH at 16q22.1. Recruitment of new families is now required to define a smaller linked region and to focus research on a reduced number of genes. Finally, our results also

show the existence of other ADH genes through the identification of 9 families unlinked to either the *LDLR*, *APOB*, *PCSK9* or the new *HCHOLA4* gene.

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Legends

Figure 1: Regional haplotype of the HCHOLA4 locus in the HC6 family.

Blackened symbols represent affected subjects, white symbols represent unaffected subjects while individuals with undetermined status are in grey.

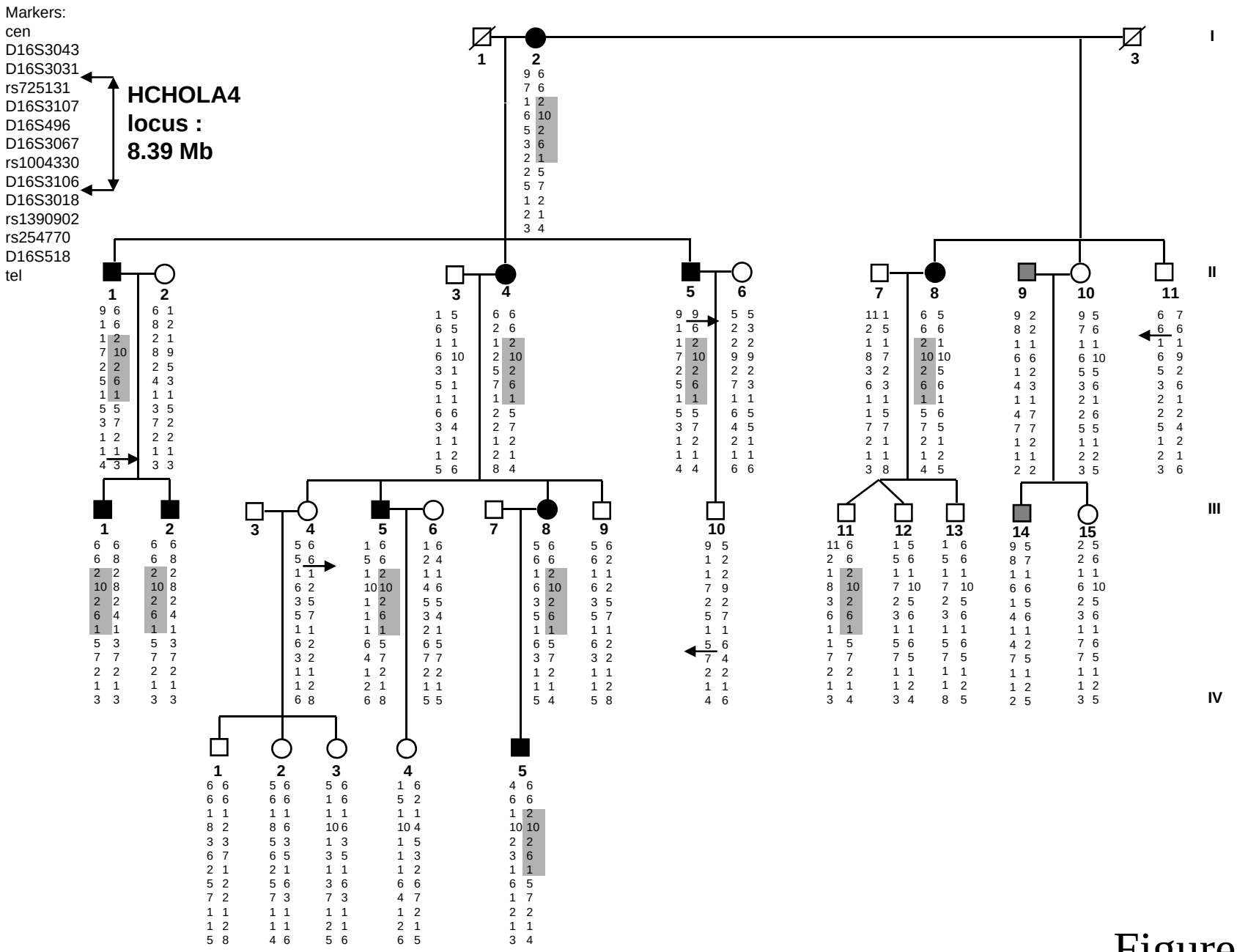
Figure 2: Multipoint LOD scores of the HCHOLA4 locus.

The parameters used for this analysis were: dominant inheritance, incomplete penetrance (0.9) and frequency of the disease-causing allele at 0.004% (SimWalk v2.91).

Table 1 : Lipids values for subjects of the HC6 family. The proband is indicated in bold. Values are given in mmol/L.

Subject	Gender	Age	Total Cholesterol	LDL-Cholesterol	Triglycerids	Status in linkage analyses
I-2	F	81	10.09	7.69	1.40	Affected
II-1	M	59	8.28	6.40	1.70	Affected
II-2	F	56	5.86	3.87	1.22	Non-affected
II-3	M	61	5.99	4.10	1.70	Non-affected
II-4	**F**	**54**	**9.39**	**7.25**	**1.09**	**Affected**
II-5	M	56	9.60	7.10	1.08	Affected
II-6	F	54	6.32	3.48	0.63	Non-affected
II-7	M	49	4.90	3.41	1.00	Non-affected
II-8	F	45	6.81	4.95	1.69	Affected
II-9	M	47	9.83	6.71	2.53	Unknown
II-10	F	44	5.39	3.59	0.50	Non-affected
II-11	M	40	5.88	4.10	0.50	Non-affected
III-1	M	28	6.32	4.75	1.31	Affected
III-2	M	26	7.17	5.55	1.01	Affected
III-4	F	36	3.41	2.09	0.91	Non-affected
III-5	M	31	7.48	5.08	2.39 *	Affected
III-6	F	34	5.78	3.79	0.50	Non-affected
III-8	F	32	7.69	5.78	1.46	Affected
III-9	M	23	4.52	2.99	1.04	Non-affected
III-10	M	30	5.60	3.46	0.89	Non-affected
III-11	M	23	4.70	3.30	0.70	Non-affected
III-12	M	23	4.28	2.99	0.90	Non-affected
III-13	M	14	3.69	2.40	0.50	Non-affected
III-14	M	16	5.88	4.41	0.99	Unknown
III-15	F	23	5.29	2.99	0.80	Non-affected
IV-1	M	14	2.99	1.60	1.00	Non-affected
IV-2	F	11	3.30	1.81	0.70	Non-affected
IV-3	F	7	2.30	0.90	1.00	Non-affected
IV-4	F	11	4.90	3.10	0.80	Non-affected
IV-5	M	2	7.35	6.24	0.98	Affected

* Non fasting.


Markers:
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D16S3031
rs725131
D16S3107
D16S496
D16S3067
rs1004330
D16S3106
D16S3018
rs1390902
rs254770
D16S518
tel
HCHOLA4
locus :
8.39 Mb
I
II
III
IV

Figure 1

Table 2 : Two-Point Parametric Linkage Analysis in the whole HC6 family.

		LOD score at θ =						
MARKER	**Position (bp)**	**0.00**	**0.05**	**0.10**	**0.15**	**0.20**	**0.25**	**0.30**
D16S3043	64 049 873 - 64 049 998	-4.82	-1.16	-0.68	-0.41	-0.25	-0.14	-0.08
D16S3031	64 338 902 - 64 339 161	-1.29	1.32	1.46	1.44	1.34	1.18	0.98
rs725131	65,143,211	**3.36**	**3.17**	2.92	2.62	2.28	1.91	1.51
D16S3107	66 215 439 - 66 215 732	**3.77**	**3.46**	**3.14**	2.81	2.45	2.07	1.67
D16S496	67 506 219 -67 506 438	1.23	1.15	1.02	0.84	0.64	0.42	0.21
D16S3067	67 666 169 - 67 666 312	**3.91**	**3.59**	**3.26**	2.91	2.54	2.14	1.73
rs904809	68,508,792	0.77	0.80	0.79	0.75	0.68	0.60	0.50
rs722579	69,085,725	0.92	0.96	0.94	0.89	0.82	0.73	0.62
rs1004330	69,590,464	1.17	1.06	0.94	0.82	0.69	0.56	0.42
rs951939	70,367,035	1.16	1.18	1.15	1.08	0.99	0.88	0.74
rs952160	70,523,226	1.83	1.80	1.71	1.59	1.44	1.27	1.07
D16S3106	70 745 261 - 70 745 460	1.62	1.51	1.34	1.13	0.88	0.62	0.36
rs811047	70,818,194	1.25	1.17	1.03	0.86	0.66	0.45	0.25
rs1383361	72,231,717	1.41	1.28	1.14	1.00	0.85	0.70	0.53
D16S512	72 625 213 - 72 625 415	0.62	0.65	0.64	0.59	0.52	0.43	0.33
D16S3018	72 730 203 - 72 730 462	1.72	1.85	1.85	1.76	1.62	1.42	1.17
rs725710	73,431,014	1.27	1.15	1.02	0.89	0.75	0.61	0.46
D16S538	74 438 373 - 74 438 918	1.76	1.58	1.39	1.20	0.99	0.79	0.59
D16S3083	75 042 911 - 75 043 109	2.41	2.20	1.99	1.76	1.52	1.27	1.01
D16S515	75 074 576 - 75 074 715	1.36	1.47	1.48	1.42	1.33	1.19	1.02
D16S3097	75 946 568 - 75 946 765	1.48	1.32	1.17	1.01	0.86	0.72	0.57
D16S3138	76 161 268 - 76 161 492	1.79	1.76	1.67	1.55	1.41	1.24	1.04
rs254770	76,435,072	-4.48	-0.67	-0.44	-0.31	-0.22	-0.16	-0.11
D16S518	76 695 528 - 76 695 807	-11.64	-1.53	-0.64	-0.18	0.08	0.22	0.27

The parameters used for this analysis were: dominant inheritance, reduced penetrance of the disease trait (0.9) and frequency of the disease-causing allele at 0.004% (SuperLink v1.5).

Figure 2

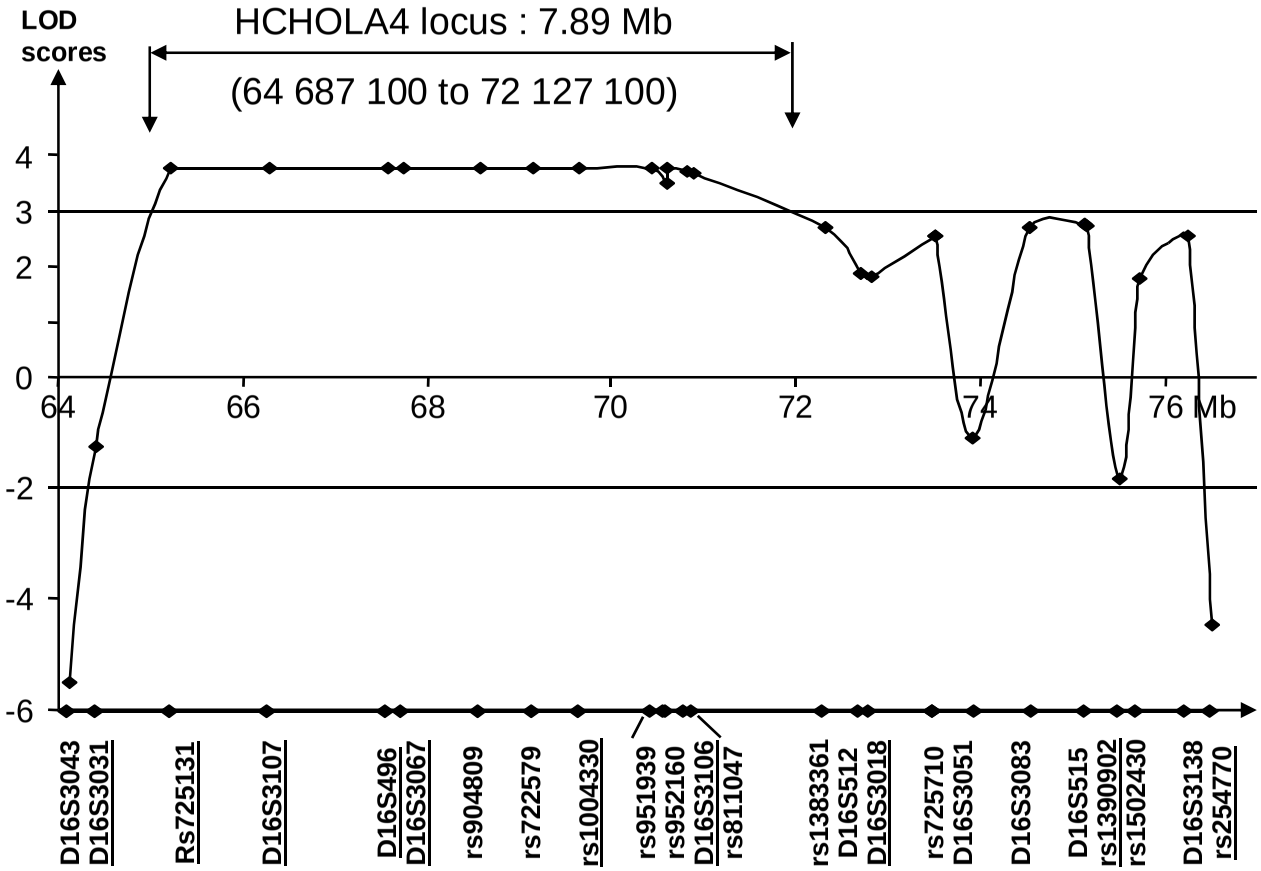
LOD scores
HCHOLA4 locus : 7.89 Mb
(64 687 100 to 72 127 100)
4
3
2
0
-2
-4
-6
64
66
68
70
72
74
76 Mb
D16S3043
D16S3031
Rs725131
D16S3107
D16S496
D16S3067
rs904809
rs722579
rs1004330
rs951939
rs952160
D16S3106
rs811047
rs1383361
D16S512
D16S3018
rs725710
D16S3051
D16S3083
D16S515
rs1390902
rs1502430
D16S3138
rs254770

Table 3 : SimWalk multipoint parametric linkage analysis of the HCHOLA4 locus in 18 families.

						SimWalk multipoint LOD (θ = 0)				
	Nb.		Nb.	ELOD		D16S3031	D16S3107	D16S3067	D16S3106	D16S3018
Familly	Subjects	Affected	meiosis	av.	max.	64,338,902	66,215,433	67,666,169	70,745,261	72,730,203
HC14	13	10	9	1.29	1.80	-7.84	-7.19	-6.85	-9.16	-6.49
HC32	4	3	2	0.12	0.26	-0.74	-0.74	-0.74	-0.74	na
HC35	5	4	3	0.45	0.60	-1.85	-2.39	-2.26	-2.71	na
HC38	4	3	2	0.23	0.30	0.30	0.30	0.30	0.30	0.30
HC42	6	4	5	0.55	0.81	-2.85	-2.29	-2.15	-2.89	-2.99
HC49	4	3	2	0.23	0.30	0.30	0.30	0.30	0.30	0.30
HC73	15	4	10	1.06	1.69	-8.18	-7.16	-6.48	-10.06	-7.29
HC120	4	3	2	0.22	0.30	0.30	0.30	0.30	0.28	0.26
HC122	8	4	5	0.70	1.12	-0.19	-0.14	-0.12	-0.10	-0.04
HC126	11	3	6	0.59	1.21	-2.21	-3.21	-3.21	-1.65	-0.62
HC136	5	4	3	0.44	0.60	-2.51	0.60	0.60	0.60	0.60
HC138	6	5	4	0.53	0.9	-6.57	-5.14	-4.95	-5.36	-4.87
HC205	6	3	4	0.50	0.86	0.86	0.86	0.86	0.86	0.86
HC218	4	3	2	0.11	0.26	-0.74	-0.74	-0.73	-0.74	-0.74
HC257	5	4	3	0.44	0.60	-1.75	-2.14	-2.13	-2.67	-1.24
HC266	5	3	3	0.33	0.56	0.56	0.56	0.56	0.56	0.56
HC374	8	5	4	0.68	0.9	-1.85	-1.80	-1.84	-2.41	-2.55
HC438	10	6	6	1.18	2.52	-4.88	-3.57	-3.26	-3.18	-8.59